5' 3' Immunostimulatory oligonucleotide

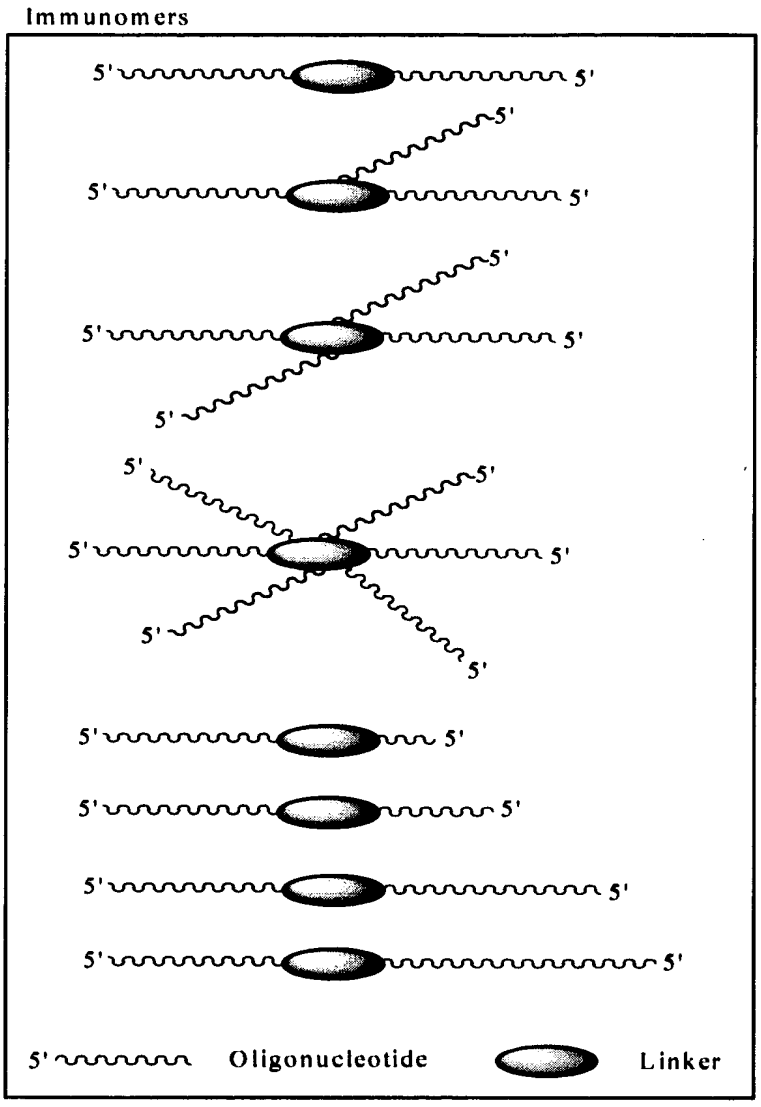
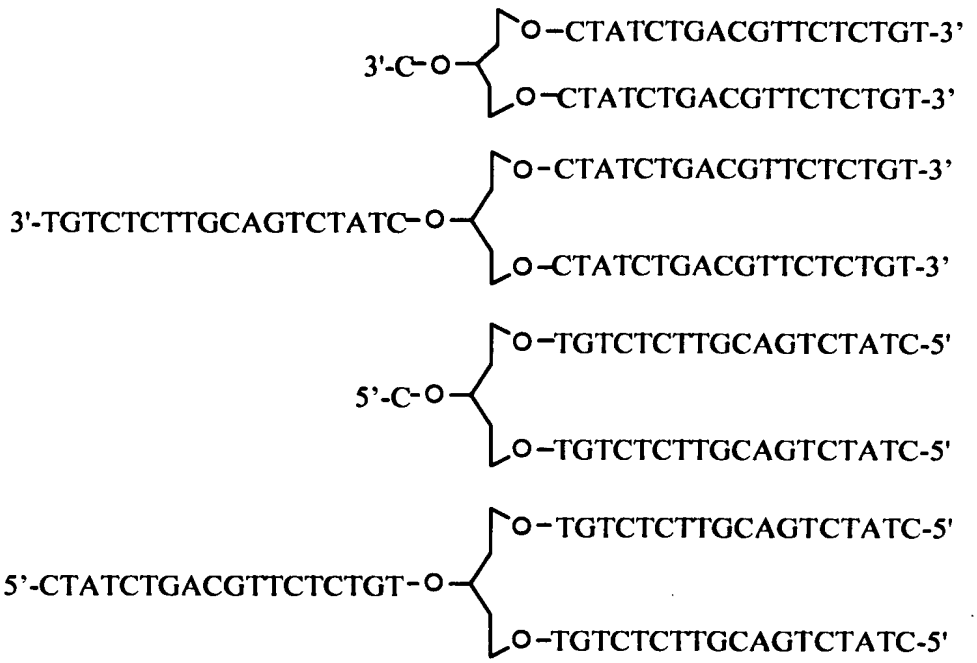


Figure 1

Figure 2


3'-C-O
O-CTATCTGACGTTCTCTGT-3'
O-CTATCTGACGTTCTCTGT-3'
3'-TGTCTCTTGCAGTCTATC-O
O-CTATCTGACGTTCTCTGT-3'
O-CTATCTGACGTTCTCTGT-3'
5'-C-O
O-TGTCTCTTGCAGTCTATC-5'
O-TGTCTCTTGCAGTCTATC-5'
5'-CTATCTGACGTTCTCTGT-O
O-TGTCTCTTGCAGTCTATC-5'
O-TGTCTCTTGCAGTCTATC-5'

Linkers for linear synthesis

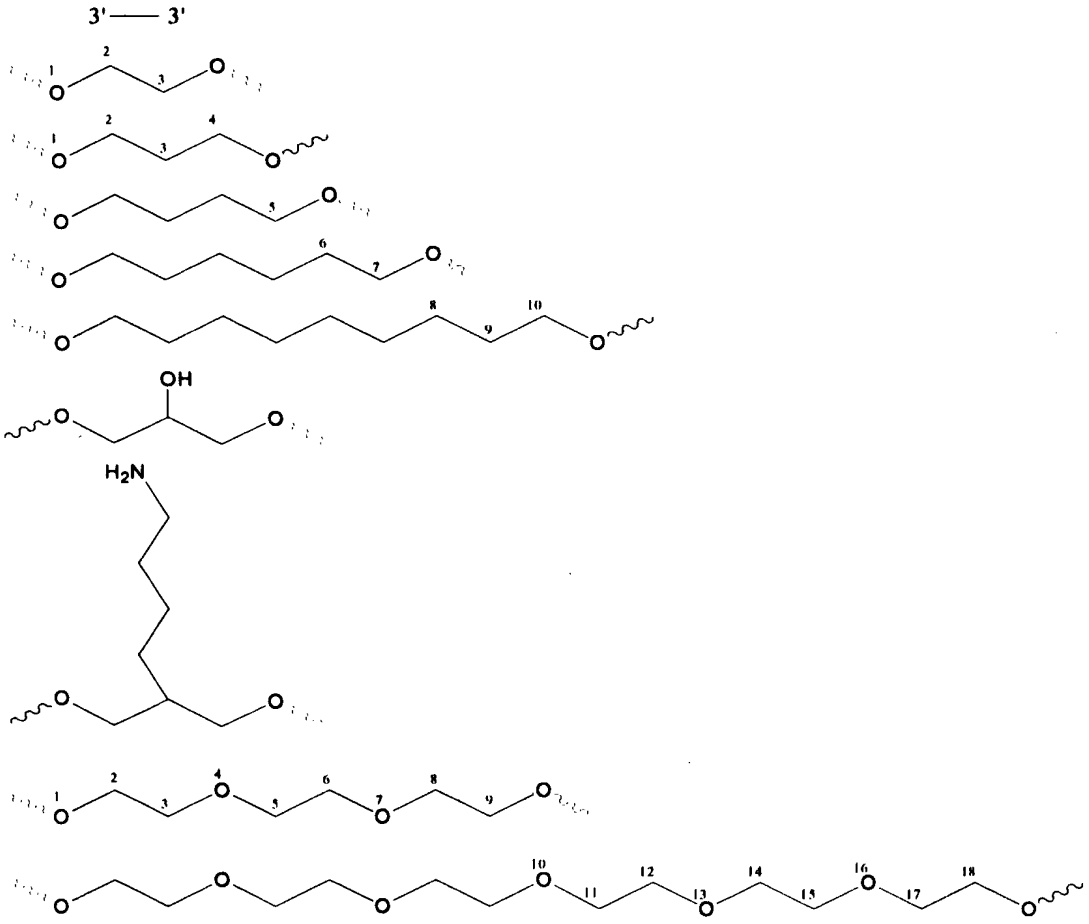
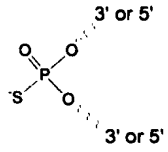
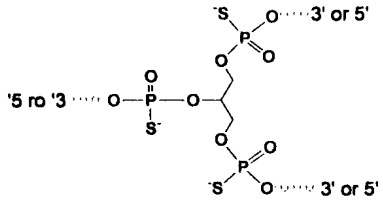


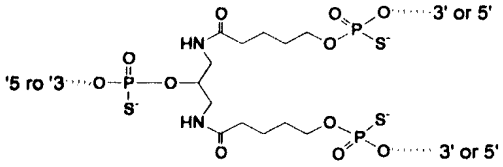
Figure 3



No linker



S, Glycerol brancher;
Short linker



B, Sym. brancher;
Long linker

Figure 4

Linear Synthesis of Immunomers

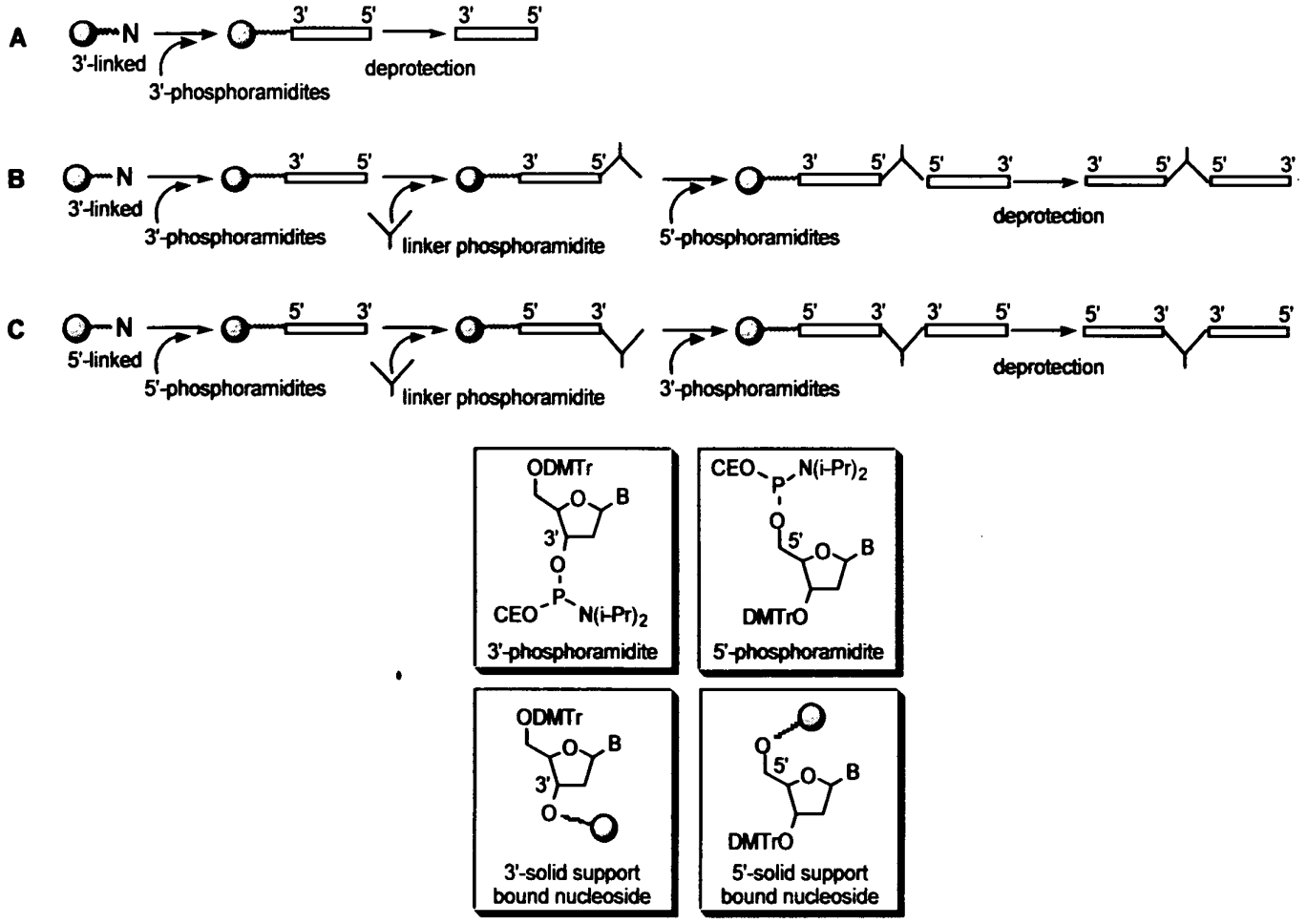


Figure 5

Figure 6

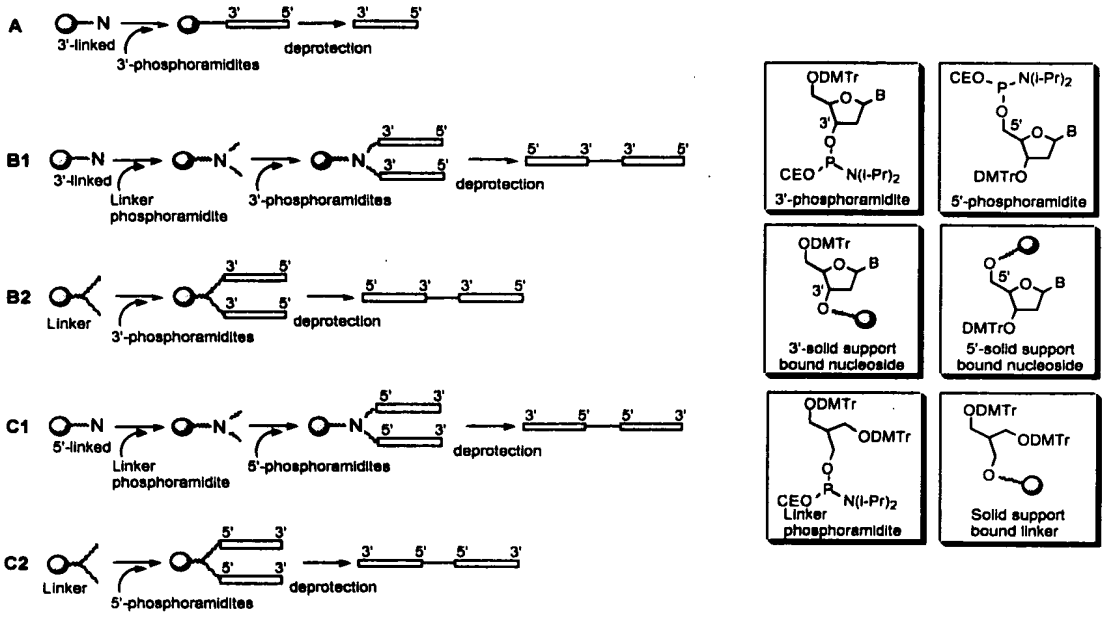

Parallel Synthesis of Immunomers
A
3'-linked
3'-phosphoramidites
deprotection
B1
3'-linked
Linker phosphoramidite
3'-phosphoramidites
deprotection
B2
Linker
3'-phosphoramidites
deprotection
C1
5'-linked
Linker phosphoramidite
5'-phosphoramidites
deprotection
C2
Linker
5'-phosphoramidites
deprotection
3'-phosphoramidite
5'-phosphoramidite
3'-solid support bound nucleoside
5'-solid support bound nucleoside
Linker phosphoramidite
Solid support bound linker

Figure 7

Figure 7A

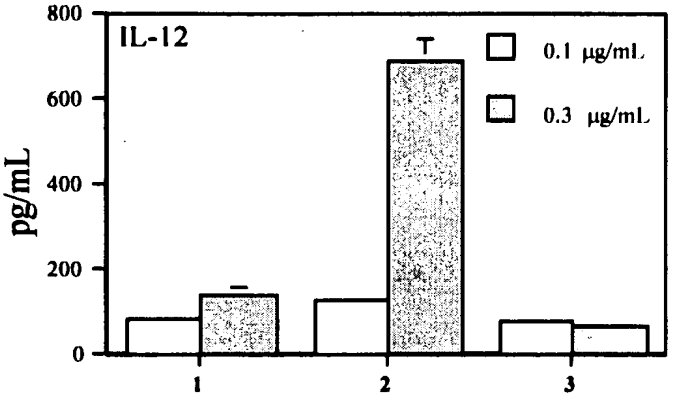


Figure 7B

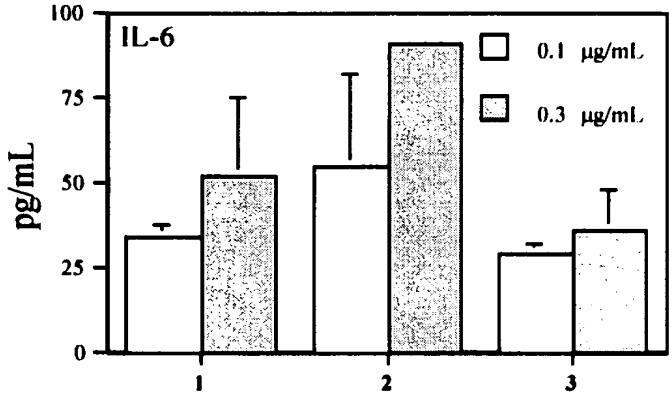


Figure 7C

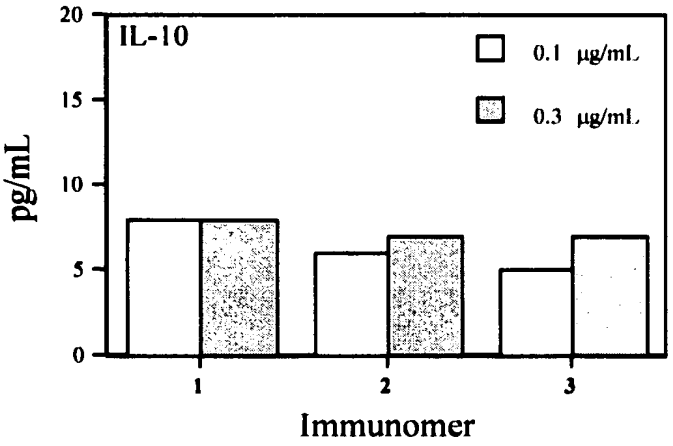


Figure 8.

Figure 8A

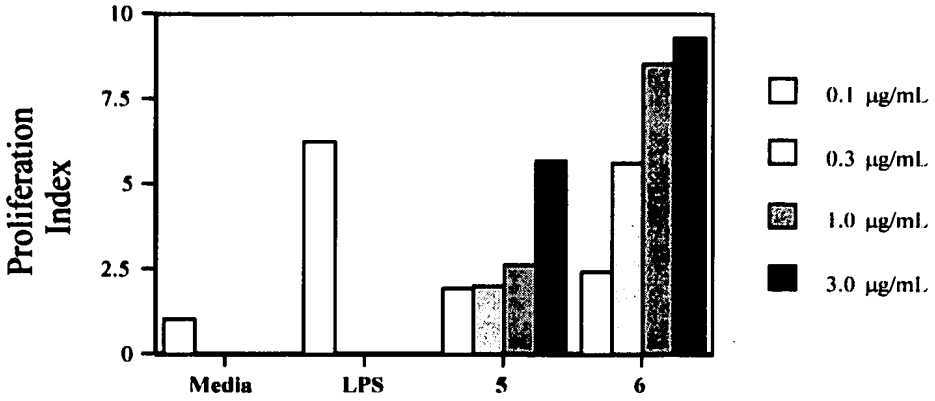


Figure 8B

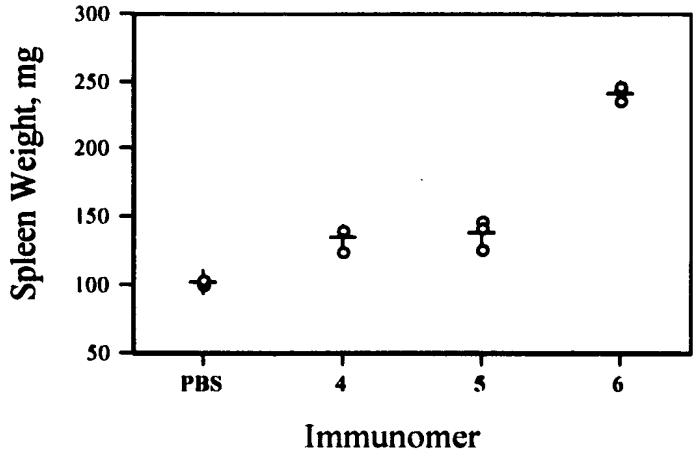


Figure 9.

Figure 9A

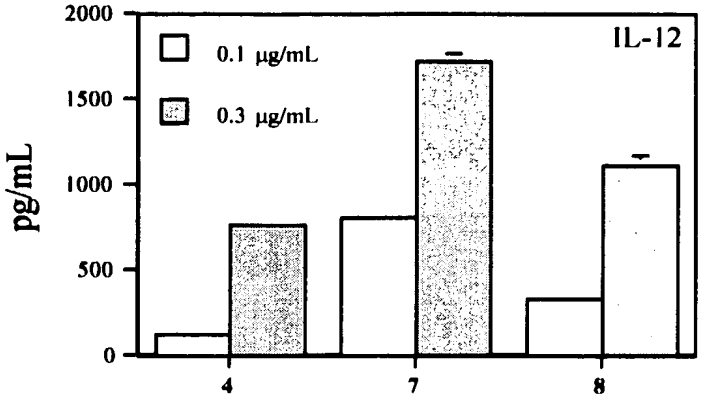


Figure 9B

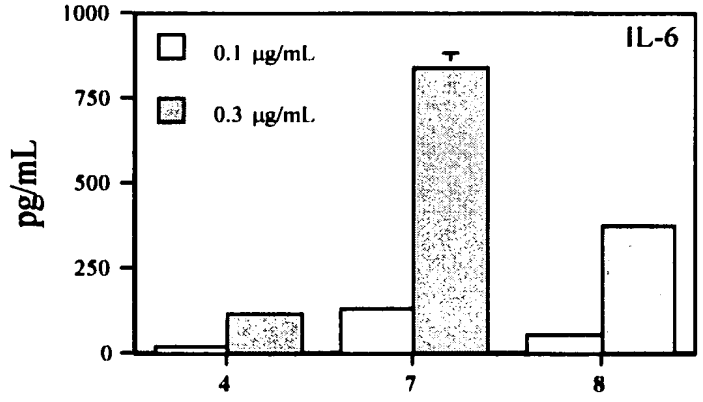


Figure 9C

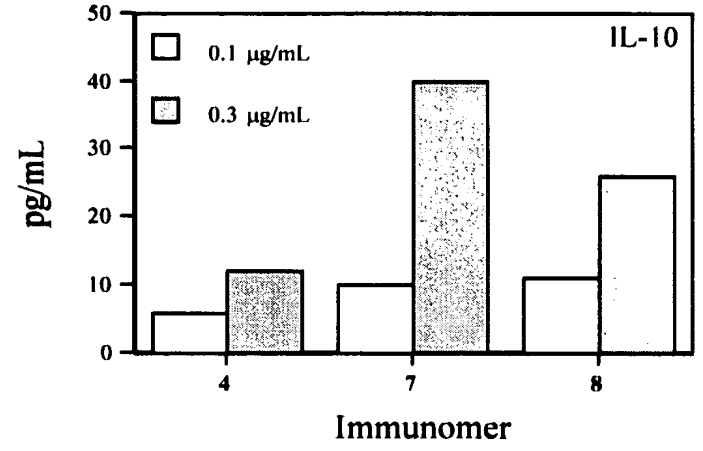


Figure 10.

Figure 10A

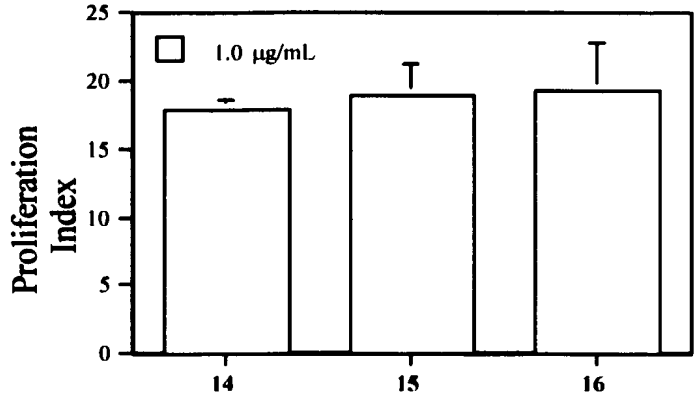


Figure 10B

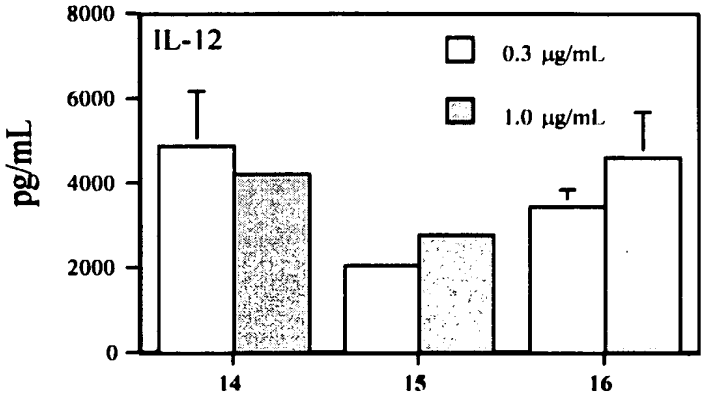


Figure 10C

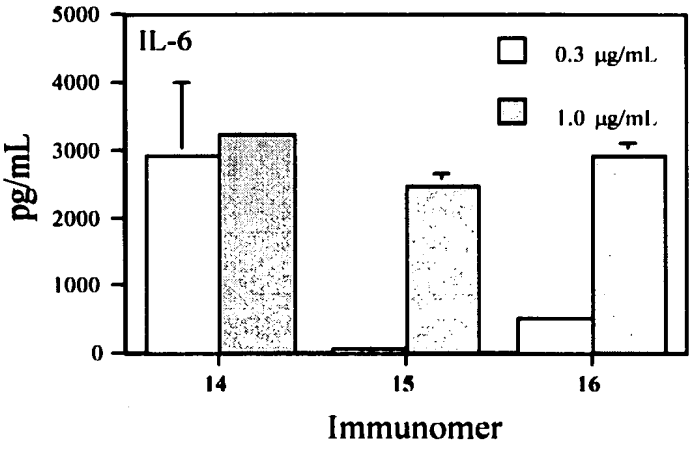


Figure 11.

Figure 11A

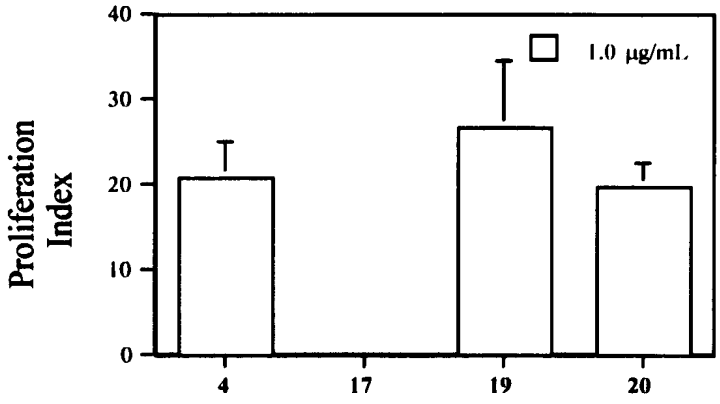


Figure 11B

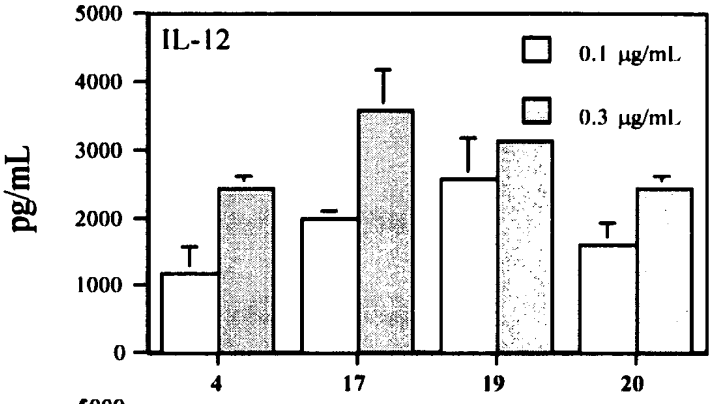


Figure 11C

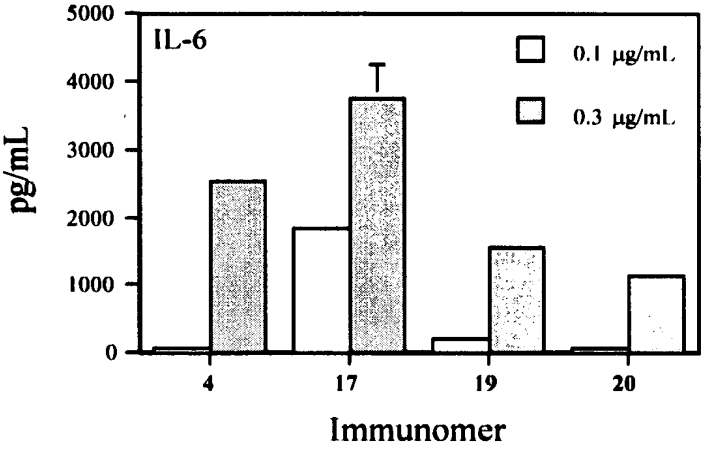
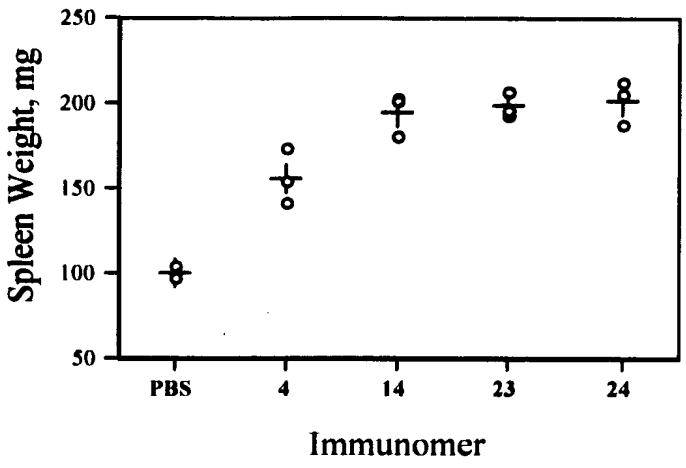


Figure 12.



Possible sites for conjugation

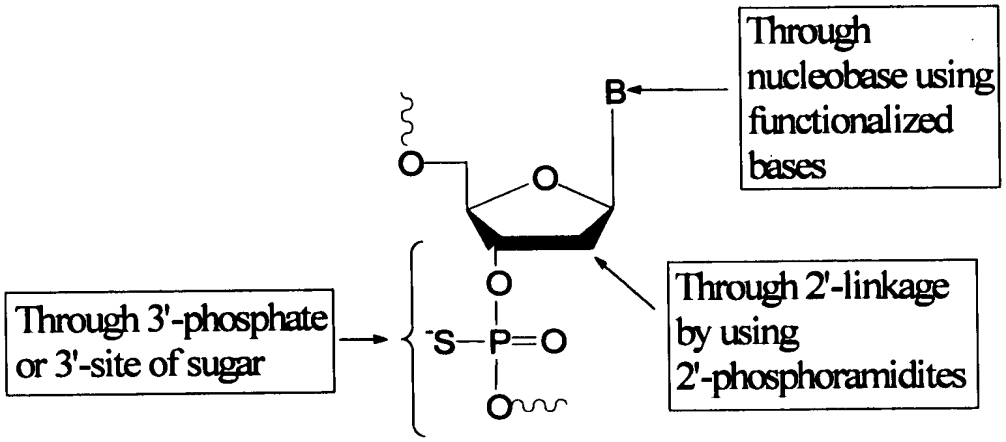
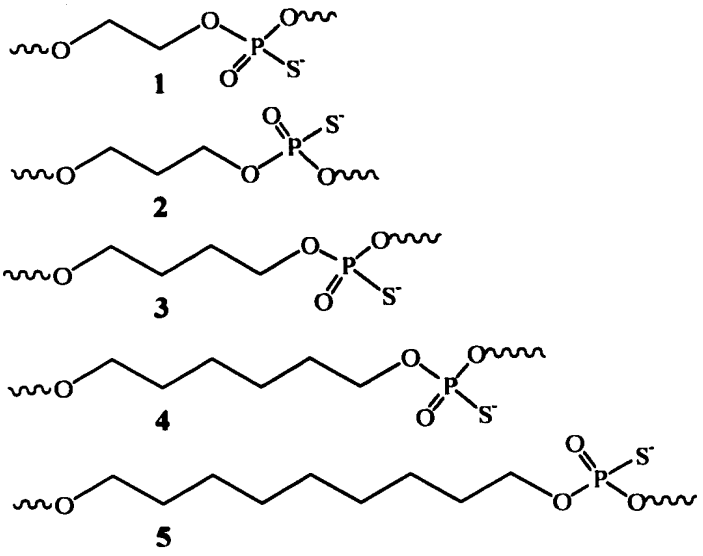
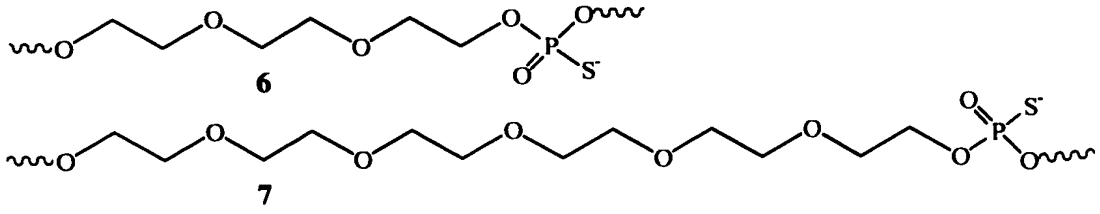


Figure 13

A. Alkyl linkers



B. Ethylene-glycol linkers



C. Branched alkyl linkers

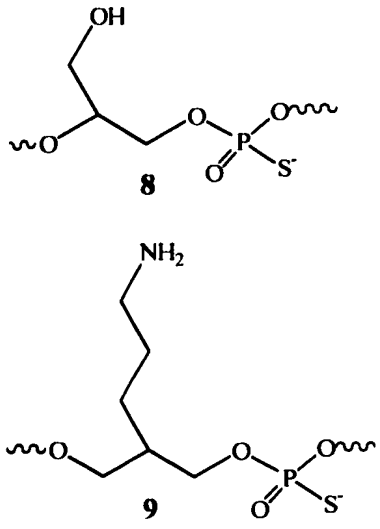


Figure 14

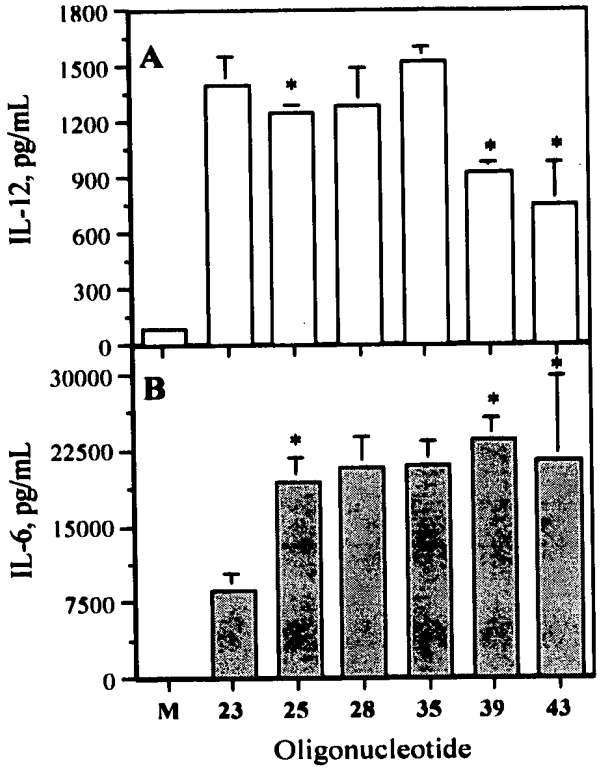

A
B
IL-12, pg/mL
IL-6, pg/mL
1800
1500
1200
900
600
300
0
30000
22500
15000
7500
0
M
23
25
28
35
39
43
Oligonucleotide

Figure 15

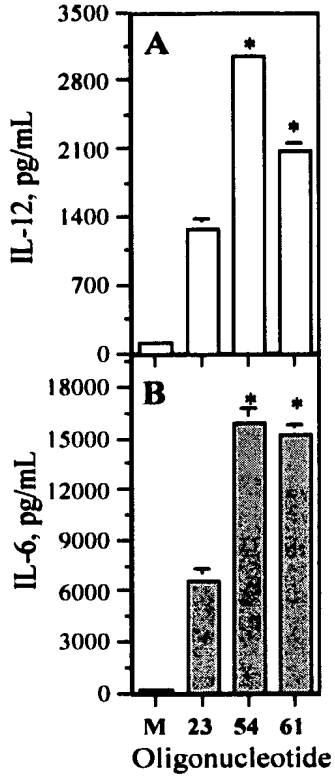

A
B
IL-12, pg/mL
IL-6, pg/mL
3500
2800
2100
1400
700
0
18000
15000
12000
9000
6000
3000
0
M
23
54
61
Oligonucleotide

Figure 16

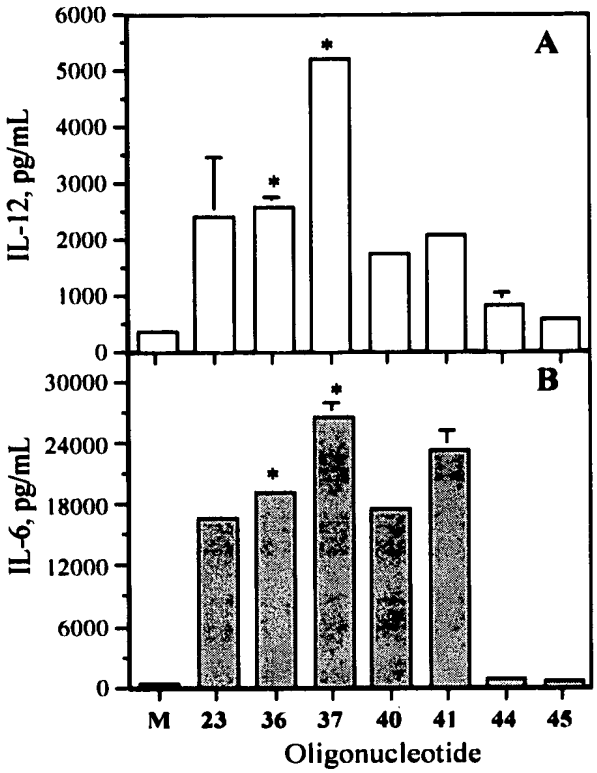

A
B
IL-12, pg/mL
IL-6, pg/mL
6000
5000
4000
3000
2000
1000
0
30000
24000
18000
12000
6000
0
M
23
36
37
40
41
44
45
Oligonucleotide

Figure 17

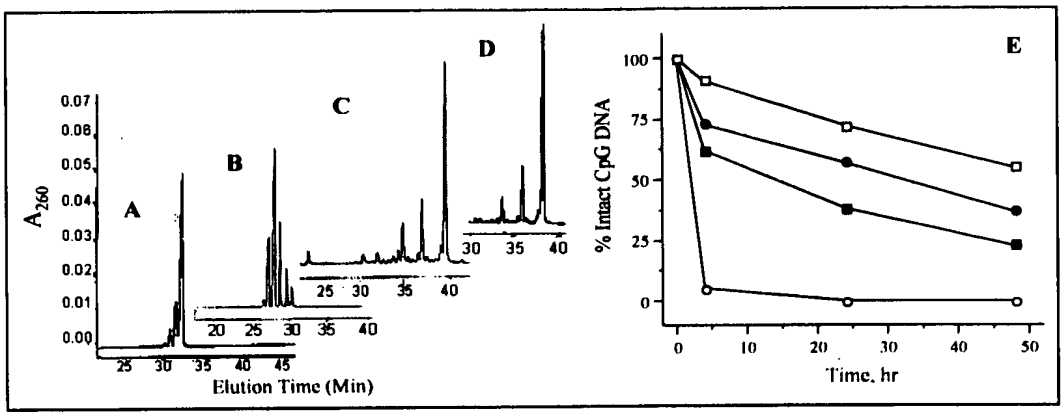


Figure 18

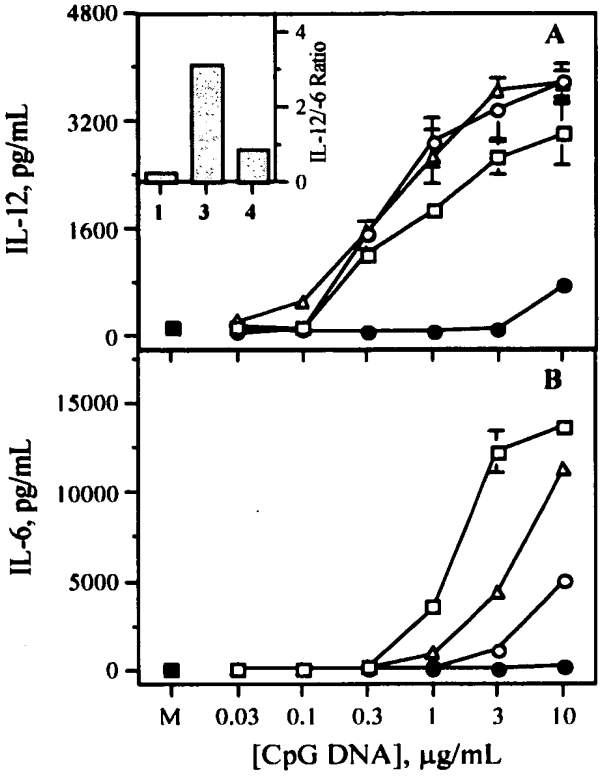

4800
3200
1600
0
IL-12, pg/mL
A
4
2
0
IL-12/-6 Ratio
1
3
4
B
15000
10000
5000
0
IL-6, pg/mL
M
0.03
0.1
0.3
1
3
10
[CpG DNA], μg/mL

Figure 19

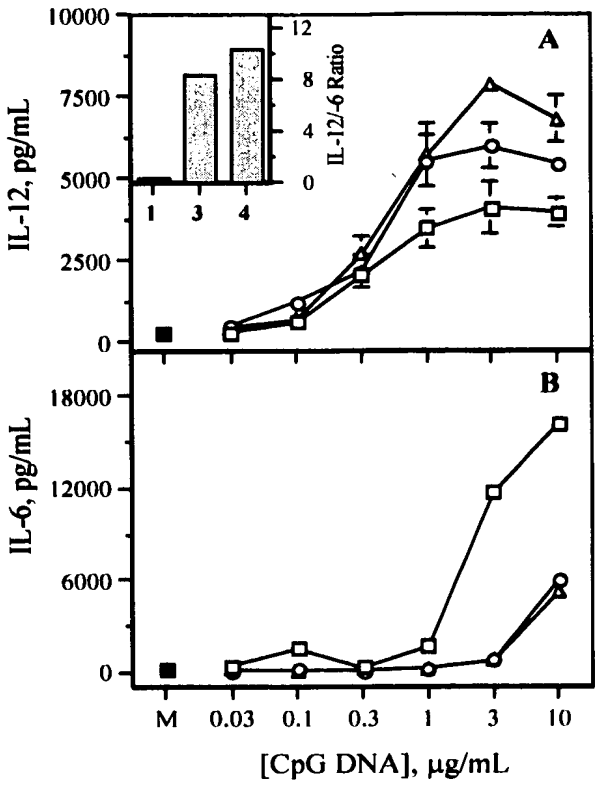

10000
7500
5000
2500
0
IL-12, pg/mL
12
8
4
0
IL-12/-6 Ratio
1
3
4
A
B
18000
12000
6000
0
IL-6, pg/mL
M
0.03
0.1
0.3
1
3
10
[CpG DNA], μg/mL

Figure 20

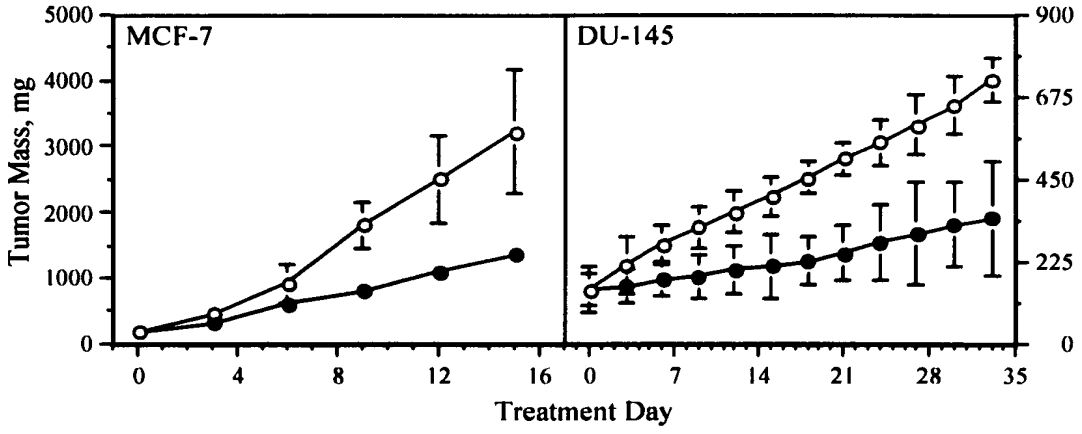
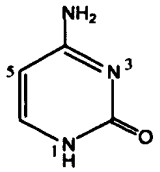
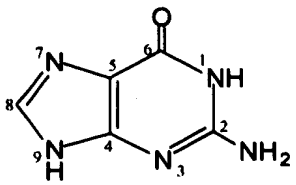

MCF-7
DU-145
Tumor Mass, mg
5000
4000
3000
2000
1000
0
900
675
450
225
0
0
4
8
12
16
0
7
14
21
28
35
Treatment Day

Figure 21

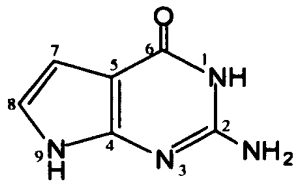
Some pyrimidine and purine structures



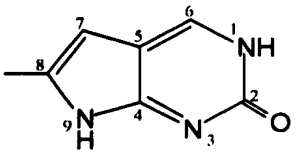
2-Oxy-4-amino pyrimidine or cytosine



2-Amino-6-oxy-purine or Guanine



2-Amino-6-oxy-7-deaza-purine or 7-Deaza-guanine



2-Oxy-7-deaza-8-methyl purine

Figure 22

4 d(5'-CTATCTGA<u>CG</u>TTCTCTGT-3')
189 d(5'-CTATCTGA<u>**RG**</u>TTCTCTGT-3')
10 d(5'-CTATCTGA<u>**CR**</u>TTCTCTGT-3')

25 d(5'-CTATCTGT<u>CG</u>TTCTCTGT-3')
190 d(5'-CTATCTGT<u>**RG**</u>TTCTCTGT-3')

191 d(5'-TCTGA<u>**RG**</u>TTCT-**L**-TCTT<u>G**R**</u>AGTCT-5')
192 d(5'-TCTGT<u>**RG**</u>TTCT-**L**-TCTT<u>G**R**</u>TGTCT-5')

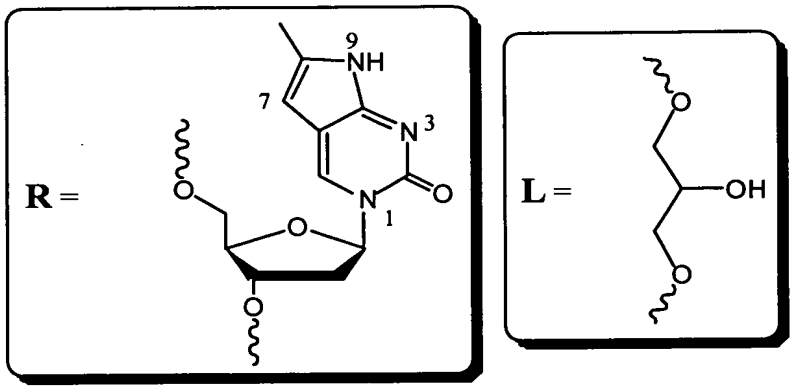
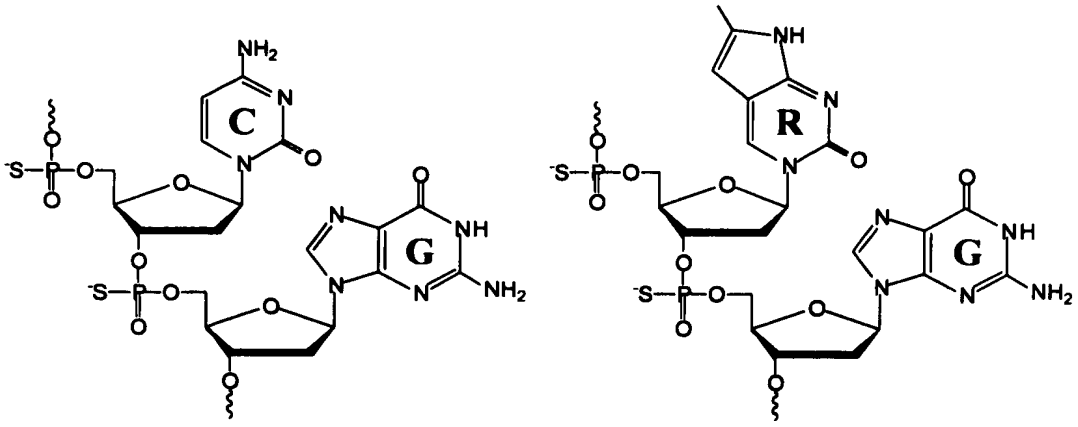


Figure 23

Comparison of Natural Pyrimidine-Purine Immunostimulatory Motif and Synthetic-Purine-Guanine Immunostimulatory Motif (RpG)



CpG RpG

Figure 24

Immunostimulatory activity of parent oligonucleotide **1** containing CpG dinucleotide motif, oligonucleotide **2** containing RpG dinucleotide motif and control oligonucleotide **3** containing GpR dinucleotide motif in mouse spleen cell culture assays. All sequences contain mouse-specific immunostimulatory motif (GACGTT).

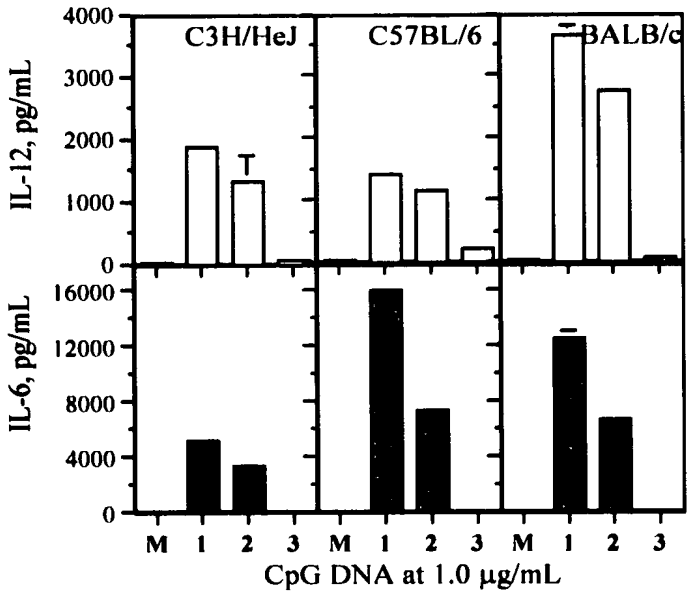


Figure 25

Immunostimulatory activity of parent oligonucleotide **4** containing CpG dinucleotide motif, and oligonucleotide **5** containing RpG dinucleotide motif in mouse spleen cell culture assays. All sequences contain human-specific immunostimulatory motif (GTCGTT).

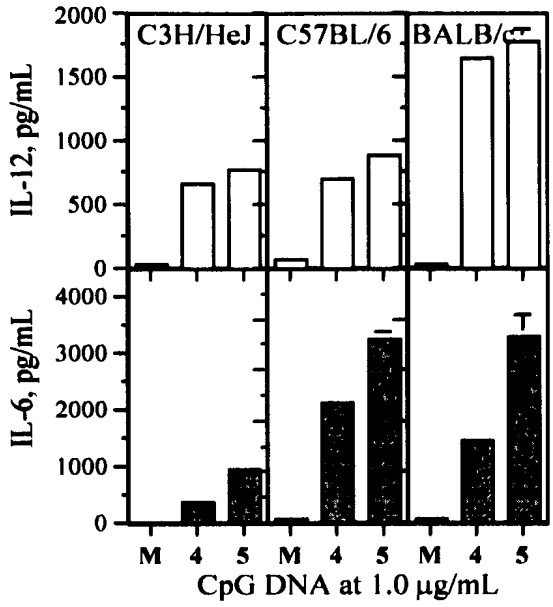


Figure 26

Immunostimulatory activity of parent oligonucleotides **1** and **4** containing CpG dinucleotide motif, and immunomers **6** and **7** containing RpG dinucleotide motif in mouse spleen cell culture assays. Sequences **1** and **6** contain mouse-specific immunostimulatory motif (GACGTT) and sequences **4** and **7** contain human-specific immunostimulatory motif (GTCGTT).

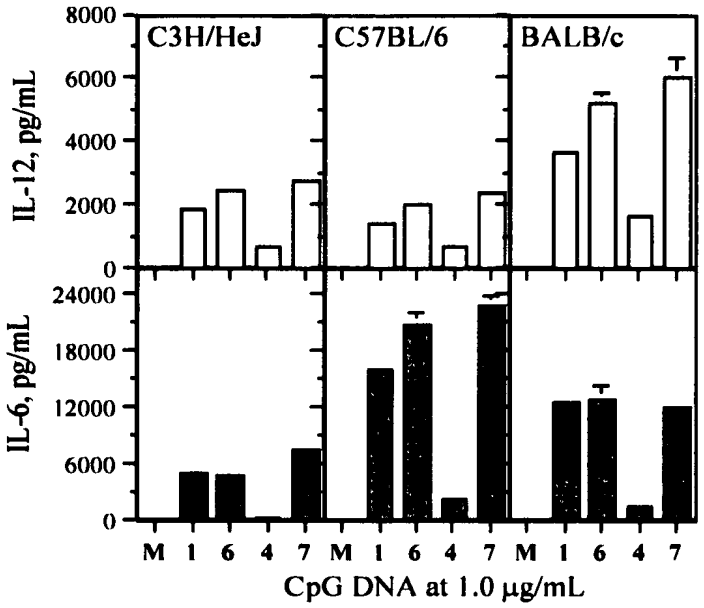


Figure 27

Immunostimulatory activity of parent oligonucleotides **1** and **4** containing CpG dinucleotide motif, and immunomers **6** and **7** containing RpG dinucleotide motif in J774, macrophage-like cell culture assays. Sequences **1** and **6** contain mouse-specific immunostimulatory motif (GACGTT) and sequences **4** and **7** contain human-specific immunostimulatory motif (GTCGTT).

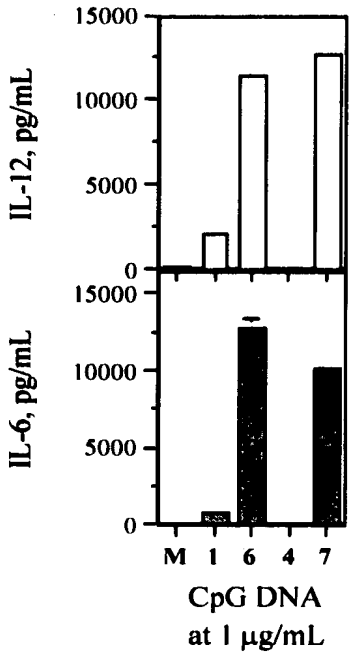


Figure 28

Activation of NF-κB and degradation of Iκ-Bα in J774 cells as a measure of immunostimulatory activity of parent oligonucleotides **1** - **7**.

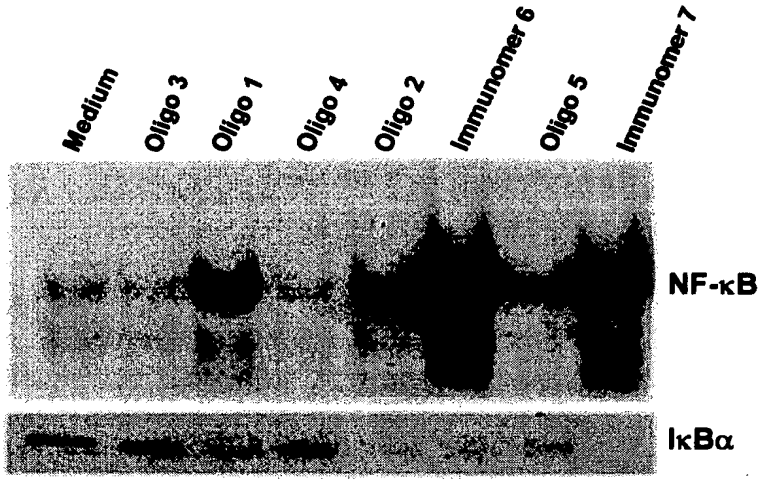


Figure 29

Immunostimulatory activity of immunomer 7 human PBMC cultures (one donor) at 10 μg/mL concentration.

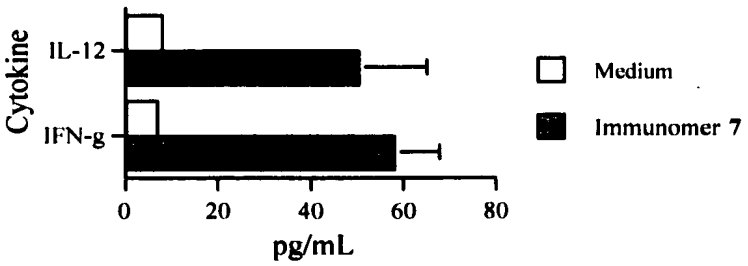


Figure 30